

# Low Nanogram Per Liter Determination of Halogenated Nonylphenols, Nonylphenol Carboxylates, and Their Non-Halogenated Precursors in Water and Sludge by Liquid Chromatography Electrospray Tandem Mass Spectrometry

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A new LC-MS-MS method for quantitative analysis of nonylphenol (NP), nonylphenol carboxylates (NPECs), and their halogenated derivatives: brominated and chlorinated nonylphenols (BrNP, ClNP), brominated and chlorinated nonylphenol carboxylates (BrNPE<sub>1</sub>C and ClNPE<sub>1</sub>C) and ethoxycarboxylates (BrNPE<sub>2</sub>C and ClNPE<sub>2</sub>C) in water and sludge has been developed. Electrospray negative ionization MS-MS was applied for the identification of above mentioned compounds. Upon collision-induced dissociation, their deprotonated molecules gave different fragments formed by the cleavage of the alkyl moiety and/or (ethoxy)carboxylic moiety. For halogenated compounds a highly diagnostic characteristic pattern of isotopic doublet signals was obtained and fragmentation yielded, in addition to above mentioned ions, [Br]<sup>-</sup> and [Cl]<sup>-</sup>, respectively. Quantitative analysis was done in the multiple reaction monitoring (MRM) mode, using two specific combinations of a precursor-product ion transitions for each compound. Additionally, for halogenated compounds two specific channels for each transition reaction, corresponding to two isotopes, were monitored and the ratio of their abundances used as an identification criterion. The method has been validated in terms of sensitivity, selectivity, accuracy, and precision and was applied to the analysis of water and sludge samples from drinking water treatment plant (DWTP) of Barcelona (Catalonia, NE Spain). Halogenated NP and NPECs were detected in prechlorinated water in concentrations up to 315 ng/L, BrNPE<sub>2</sub>C being the most abundant compound. In the DWTP effluent non-halogenated compounds were detected at trace levels (85, 12 and 10 ng/L for NP, NPE<sub>1</sub>C, and NPE<sub>2</sub>C, respectively), whereas concentration of halogenated derivatives never exceeded 10 ng/L. Nonylphenol, brominated and chlorinated NPs were found in flocculation sludge in concentrations of 150, 105, and 145 µg/kg, respectively. Acidic polar metabolites were found in lower concentrations up to 20 µg/kg. (J Am Soc Mass Spectrom 2003, 14, 516–527) © 2003 American Society for Mass Spectrometry

**N**on-ionic surfactants, nonylphenol polyethoxylates (NPEOs), have been widely used in the last 40 years as detergents, emulsifiers, dispersants, antifoamers and pesticide adjuvants. The biodegradation of NPEOs under aerobic conditions yields mainly short ethoxy chain oligomers (NPEO<sub>1</sub> and NPEO<sub>2</sub>), whereas under anaerobic conditions fully de-

ethoxylated nonylphenol (NP) is also formed. Further transformation leads to acidic metabolites formed by oxidation of the ethoxy chain (nonylphenol carboxylates; NPECs) as well as oxidation of the branched alkyl chain [1–4]. During the chlorination process at drinking water treatment plants (DWTP) [5–8] and wastewater treatment plants (WWTP) [9, 10] the formation of halogenated derivatives, such as ring-brominated and chlorinated NPEOs, NPECs, and NPs, have been reported.

Toxicity of NP and short ethoxy chain NPEOs to aquatic organisms [11], lipophilic properties that lead to bioaccumulation in aquatic food chain [12], and ability

Published online April 2, 2003

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to mimic endogenous hormone  $17\beta$ -estradiol [13, 14] are well documented. However, little is known about environmental significance and toxicology of brominated and chlorinated alkylphenolic compounds. Maki et al. [15] determined that, both BrNPEOs and BrNPECs, show higher acute toxicity to *Daphnia magna* than their non-brominated precursors NPEOs and NPECs. A recent study, employing recombinant yeast assay (RYA) and enzyme linked receptor assay (ELRA) for the determination of estrogenic and anti-estrogenic activity, showed that halogenated compounds retained a significant affinity for the estrogen receptors suggesting that they may be still able to disturb the hormone imbalance of exposed organisms [16]. This was especially clear for halogenated NPECs, which acted as true anti-estrogens in the RYA.

The presence of alkylphenolic compounds in the environment has become of increasing concern globally and efforts have been made to determine their concentration levels in WWTP and in aquatic environments. However, studies to date have largely focused on short chain NPEOs, NPECs, and NPs, while fewer reports have included halogenated metabolites. One of the reasons for this is the low relative abundance of these compounds (generally less than 10% of the total pool of alkylphenolic compounds) and unavailability of appropriate analytical methods for their identification and quantification.

The first attempts to analyse halogenated alkylphenolic compounds were carried out using Fast Atom Bombardment-Mass Spectrometry (FAB-MS) [5, 7, 8, 17], which proved to be a reliable tool for the identification of halogenated metabolites in raw and drinking water, but not for their quantification. Recently, some efforts have been made to quantify these compounds in complex environmental and wastewater samples. Methods applied included gas chromatography-mass spectrometry (GC-MS) [10, 18, 19] (after appropriate derivatization) and reversed-phase liquid chromatography with electrospray mass spectrometry (LC-ESI-MS) [6, 20, 21]. ESI permitted the direct determination of the full range of halogenated NPEOs metabolites (i.e., XNPEOs, XNPECs and XNPs), as well as their precursors in aqueous and solid samples, thus obviating the necessity to methylate them. However, using "soft ionization" LC-MS, under conditions giving solely molecular ions, the identification of halogenated compounds is difficult since the chlorinated derivatives (CINPEO<sub>n</sub> and CINPE<sub>n</sub>C) have the same molecular mass as brominated compounds with one ethoxy group less (BrNPEO<sub>n-1</sub> and BrNPE<sub>n-1</sub>C) [6]. Moreover, in the analysis of real-world samples CINPE<sub>1</sub>C was obstructed by a severe isobaric interference of linear alkylbenzene sulfonate (C<sub>11</sub>LAS), which is often found in environmental and wastewater samples in concentrations several orders of magnitude higher than those of halogenated alkylphenolic compounds.

Thus, to obviate the matrix interference and interference of known and unknown compounds that may

cause deviations when only a single stage of mass selectivity is used, more selective methods, such as tandem mass spectrometry are needed. However, although considered as one of the most powerful techniques for structure interpretation and quantification, LC-MS-MS has been seldom used in the analysis of acidic and neutral metabolites of NPEOs [2, 22], and has not been applied thus far to study their halogenated derivatives.

In the present work, a tandem mass spectrometric investigation of halogenated NPECs, NPs and their precursors (non-halogenated analogs) was carried out. Electrospray negative ionization MS-MS was applied for the identification of acidic and neutral NPEOs metabolites. From the observed ion fragmentation pathways a reliable and sensitive quantification method, that overcomes the main drawbacks on existing methods, is developed. The method was applied to study occurrence of halogenated alkylphenolic compounds derived from chlorination treatment in DWTP of Barcelona (Spain). To our knowledge this is the first LC-MS-MS method that permits analysis of halogenated NPs and NPECs at low nanogram per liter level.

## Experimental

### Standards and Reagents

NPE<sub>1</sub>C and NPE<sub>2</sub>C were synthesized according to the method described elsewhere [19]. Technical grade 4-NP and 4-nonyloxy benzoic acid, used as an internal standard was obtained from Aldrich (Milwaukee, WI).

BrNP was synthesized using elemental bromine according to the method described by Reinhard et al. [9]. CINP was prepared by chlorination of nonylphenol using sulfuryl chloride according to the method of Stokker et al. [23]. BrNPE<sub>1</sub>C and CINPE<sub>1</sub>C were synthesized by reacting brominated and chlorinated NP, respectively, with chloroacetic acid in the presence of sodium hydride and dimethylformamide as a solvent. These two synthesized compounds rendered BrNPEO<sub>1</sub> and CINPEO<sub>1</sub> by reduction with lithium aluminum hydride in ether solution. BrNPEO<sub>2</sub> and CINPEO<sub>2</sub> were synthesized by reacting BrNP and CINP, respectively, with 2-(2-chloroethoxy)ethanol in the presence of NaOH in water. Finally, BrNPE<sub>2</sub>C and CINPE<sub>2</sub>C were obtained from BrNPEO<sub>2</sub> and CINPEO<sub>2</sub>, respectively, by oxidation with Jones reagent [9].

### Water Samples

Raw water entering the DWTP Sant Joan Despí (Barcelona, Spain) and water samples after each treatment step (i.e., prechlorination, rapid sand filtration, groundwater dilution, ozonation, granulated active carbon filtration, and final chlorination) were collected as grab samples in Pyrex borosilicate amber glass containers, previously rinsed with high-purity water.

NP, NPECs, and their halogenated derivatives were

**Table 1.** List of MRM channels, cone and collision energies applied to ESI-MS-MS for the analysis of nonylphenol, nonylphenol carboxylates, and their halogenated derivatives

Compound	MRM 1 (Quantification)			MRM 2 (Confirmation)			Ratio MRM1/ MRM2
	Precursor ( <i>m/z</i> ) → Product ( <i>m/z</i> )	Cone (V)	Collision (eV)	Precursor ( <i>m/z</i> ) → Product ( <i>m/z</i> )	Cone (V)	Collision (eV)	
NP	219 → 133	30	30	219 → 147	30	30	9.2
BrNP	297 → 79	30	30	297 → 211	30	30	12.3
	299 → 81			299 → 213			
CINP	253 → 167	30	30	253 → 181	30	30	10.5
	255 → 169			255 → 183			
NPE <sub>1</sub> C	277 → 219	10	30	219 → 133	30	30	1.8
BrNPE <sub>1</sub> C	355 → 297	10	30	355 → 79	30	30	1.0
	357 → 299			357 → 81			
CINPE <sub>1</sub> C	311 → 253	10	30	253 → 167	30	30	3.5
	313 → 255			255 → 169			
NPE <sub>2</sub> C	219 → 133	30	30	321 → 219	10	30	1.2
BrNPE <sub>2</sub> C	297 → 79	30	30	399 → 297	5	20	2.0
	299 → 81			401 → 299			
CINPE <sub>2</sub> C	253 → 167	30	30	355 → 253	5	20	5.5
	255 → 169			357 → 255			

isolated from water samples using solid-phase extraction (SPE). A more detailed description of the SPE method is given elsewhere [6]. Briefly, 500 mL of water samples were loaded onto preconditioned Accubond C18 cartridges (J and W Scientific, Folsom, CA). Cartridges were air-dried under vacuum, and were eluted with  $2 \times 4$  mL of methanol. The eluates were taken gently to dryness under a nitrogen stream and reconstituted in 500  $\mu$ L of methanol.

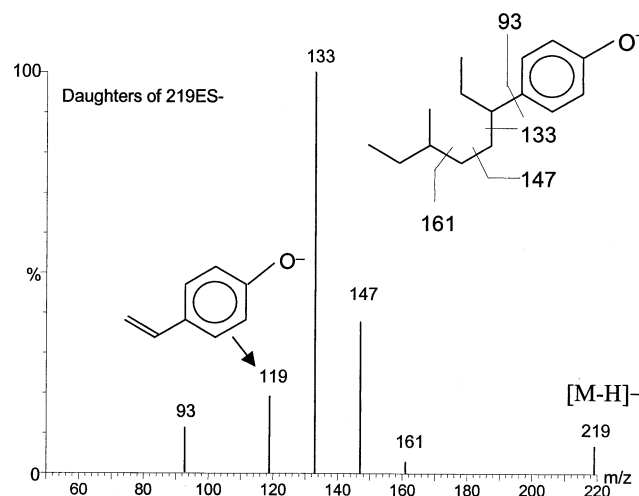
### Sludge Samples

Sludge from DWTP of Barcelona, obtained from pre-chlorinated raw water after flocculation with aluminium sulfate and mixed in a minor proportion with sludge coming from the washing of sand filters, was collected in precleaned amber glass bottles. The suspension (concentration of dry matter 3.5 to 5 g/L) was centrifuged at 4500 rpm, and the solid matter was separated and frozen at  $-20^\circ\text{C}$  before being freeze-dried.

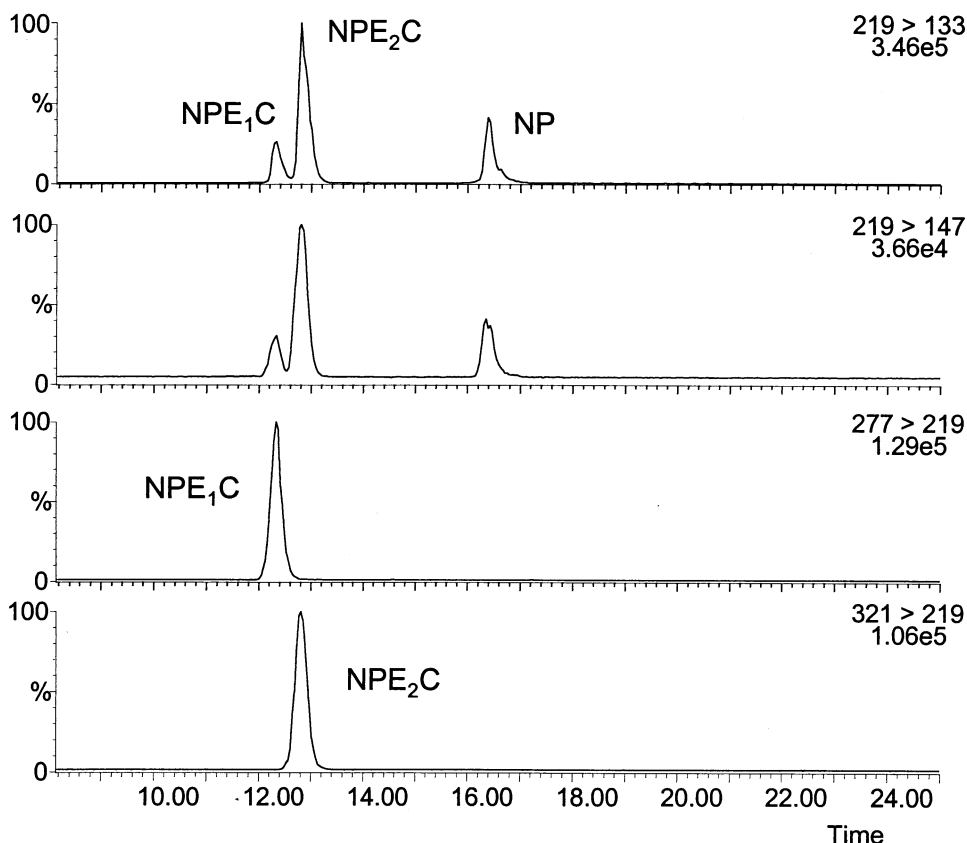
Pressurized liquid extractions (PLE) were carried out using a Dionex ASE 200 (Dionex, Idstein, Germany) as described elsewhere [24]. Briefly, 1 g sub-sample of freeze-dried sludge was mixed with  $\text{Na}_2\text{SO}_4$  and filled into 11 mL extraction cells. Extraction was carried out with acetone/methanol (1:1, vol/vol) under following conditions: temperature of  $75^\circ\text{C}$ , pressure 1500 psi, heating time 5 min, two cycles of static extraction (5 min). As a final step, the cell was purged with gaseous nitrogen. The total volume of extract was  $\sim 20$  mL. Extracts obtained by PLE, were concentrated to an approximate volume of 1 mL using a rotary vacuum, redissolved in 100 mL of HPLC water and subsequently purified by SPE using LiChrolute C18 cartridges (Merck, Darmstadt, Germany), as described elsewhere [6].

### Chromatographic Conditions

Analyses were performed on a Waters 2690 series Alliance HPLC (Waters, Milford, MA) with a quaternary pump equipped with a 120 vial capacity sample management system. The analytes were separated on a narrow-bore  $3 \mu\text{m}$ ,  $55 \times 2$  mm i.d. C<sub>18</sub> reversed phase column Purospher STAR RP-18 endcapped (Merck, Darmstadt, Germany). The sample injection volume was set at 10  $\mu\text{L}$ . A binary mobile phase gradient with methanol (A) and water (B) was used for analyte separation at a flow rate of 200  $\mu\text{L}/\text{min}$ . The elution gradient was linearly increased from 30% A to 85% A in 10 min, then increased to 95% A in 10 min and kept isocratic for 5 min.



**Figure 1.** Product ion scan of NP with *m/z* 219 and the proposed fragmentation scheme under CID conditions obtained using argon as collision gas at collision energy of 40 eV. Note: The exact branching of the alkyl chain is unknown and the alkyl isomer shown here is just one of several possibilities.



**Figure 2.** MS/MS chromatograms of raw effluent (river water) treated in Barcelona drinking water treatment plant corresponding to nonylphenolic compounds (MRM mode: 219  $\rightarrow$  133 and 219  $\rightarrow$  147 for detection of NP, NPE<sub>1</sub>C, and NPE<sub>2</sub>C; 277  $\rightarrow$  219 for NP<sub>1</sub>EC and 321  $\rightarrow$  219 for NP<sub>2</sub>EC).

### Mass Spectrometry

A bench-top triple quadrupole mass spectrometer Quattro LC from Micromass (Manchester, UK) equipped with a pneumatically assisted electrospray probe and a Z-spray interface was used for this study. Capillary voltage was set at  $-2.8$  kV, extractor lens  $7$  V and RF lens  $0.6$  V. The source and desolvation temperatures were  $150$  and  $350$   $^{\circ}\text{C}$ , respectively. The nitrogen (99.999% purity) flows were optimized at  $50$  L/h for the cone gas and  $540$  L/h for desolvation gas. For each analyte the values of the voltages applied to the cone, focusing lenses, collision cell, and quadrupoles were optimized by continuous infusion of a standard solution ( $1$   $\mu\text{g/mL}$ ) via a syringe infusion pump Kd Scientific 100 (Boston, MA) at a constant flow-rate of  $20$   $\mu\text{L/min}$ . All ESI mass spectral data were acquired with Masslynx NI software (version 3.5).

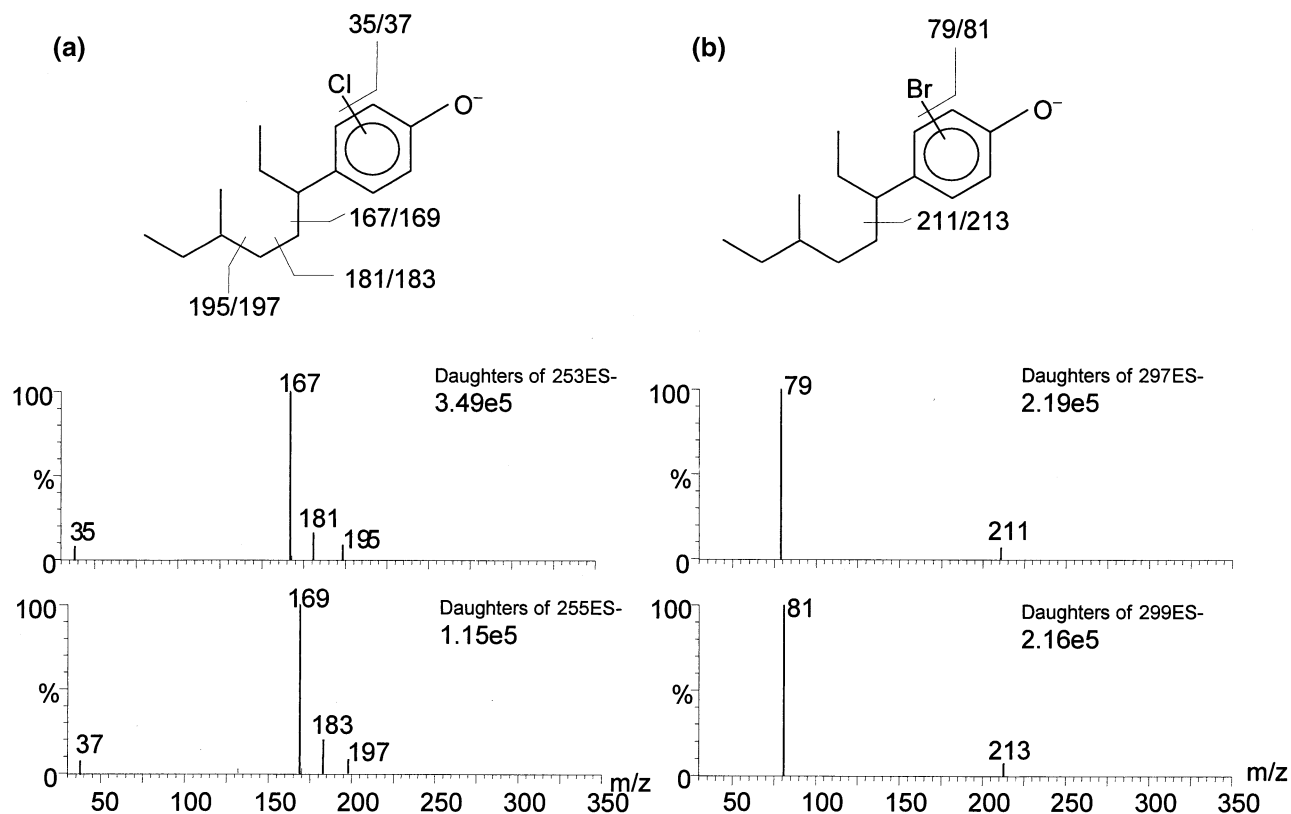
**MS scans.** For one stage MS scans the cone voltage was varied from  $-10$  to  $-50$  V according to the type of experiment performed and analyte studied. Full-scan mass spectra were recorded between  $m/z$   $30$  and  $500$ , with scan duration of  $1$  s/scan and an interscan time of  $0.1$  s.

**MS/MS scans.** The cone voltage was set to a value, which resulted in maximum abundance of the pseudo molecular ion (see Table 1). The argon collision gas was maintained at a pressure of  $5.8 \times 10^{-3}$  mbar. The optimum collision energy was chosen after performing MS/MS product ion scans on  $[\text{M} - \text{H}]^{-}$  over a range of energies between  $10$  and  $50$  eV. The electron multiplier was set at  $600$  V. For experiments performed in MRM mode scan time was  $1$  s/scan, and the dwell time ranged from  $50$  to  $200$  ms, depending on the number of transition channels monitored (from  $10$  to  $20$ ).

### Quantification

Quantitative analyses were done in MRM mode. The extent of ion suppression of MS signal was determined using 4-nonyloxy benzoic acid as an internal standard. The results (see Discussion) showed very limited signal reduction (less than  $15\%$  for sludge and negligible for water samples), thus the quantification was performed using external calibration.

Initially, a series of injections of target compounds in the concentration range from  $1$  ng/mL to  $10$   $\mu\text{g/mL}$  was used to determine the linear concentration range. Calibration curves were generated using linear regres-



**Figure 3.** CID spectra and the proposed fragmentation pattern of (a) CINP (precursor ions  $m/z$  253 and 255) and (b) BrNP (precursor ions  $m/z$  297 and 299) obtained at collision energy of 30 eV. Note: The exact branching of the alkyl chain is unknown and the alkyl isomer shown here is just one of several possibilities.

sion analysis and over the established concentration range (0.01–1  $\mu\text{g/mL}$ ) gave good fits ( $r^2 > 0.990$ ). Five-point calibration was performed daily, and the possible fluctuation in signal intensity was checked by injecting a standard solution at two concentration levels after each 8–10 injections.

## Results and Discussion

### Mass Spectrometry—Optimization of Experimental Conditions

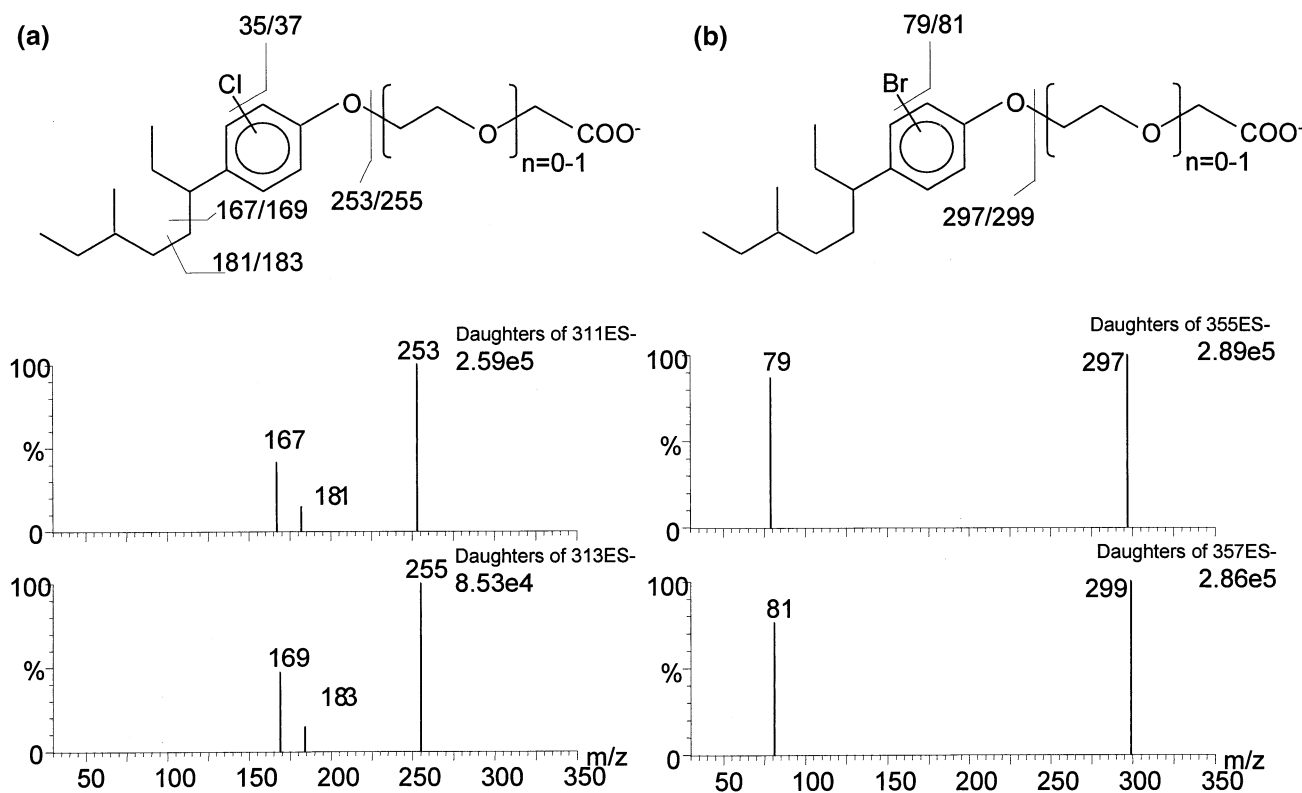
A preliminary study was carried out using the single quadrupole mode under full-scan conditions and the negative ionization. The cone voltage was adjusted to give the maximum abundance of deprotonated molecule  $[\text{M} - \text{H}]^-$ , which were chosen as precursor ions in further MS-MS experiments, performed with the purpose of finding the best instrumental conditions for the identification of target compounds.

**NP and NPECs.** The product ion scan of  $[\text{M} - \text{H}]^-$  for NP evidenced fragmentation of the side chain of deprotonated molecule (Figure 1), resulting in sequential loss of  $\text{CH}_2$  groups ( $m/z$  14), down to specie with  $m/z$  93. The most abundant fragments with  $m/z$  133 and 147 resulted from the loss of  $\text{C}_6\text{H}_{14}$  and  $\text{C}_5\text{H}_{12}$ , respectively.

The product ion spectra of deprotonated molecule at  $m/z$  277 (for  $\text{NPE}_1\text{C}$ ) and  $m/z$  321 (for  $\text{NPE}_2\text{C}$ ), showed the intense signal at  $m/z$  219, corresponding to  $[\text{M} - \text{CH}_2\text{COO} - \text{H}]^-$  and  $[\text{M} - \text{CH}_2\text{CH}_2\text{OCH}_2\text{COO} - \text{H}]^-$ , respectively, as reported previously by other authors [2, 22]. Additional fragments at  $m/z$  133 and 147 were formed by the fragmentation on the side chain, as described above for NP. Thus, specific transitions at  $m/z$  277  $\rightarrow$  219 and  $m/z$  321  $\rightarrow$  219 could be used to monitor  $\text{NPE}_1\text{C}$  and  $\text{NPE}_2\text{C}$ , respectively, while MRM channels at  $m/z$  219  $\rightarrow$  133 and  $m/z$  219  $\rightarrow$  147 are characteristic for both NP and NPECs, and could be used to monitor all these compounds: However, in the latter case good chromatographic separation is essential, as depicted in Figure 2.

**Halogenated NP and NPECs.** Owing to the presence of chlorine and bromine atoms in the molecules, halogenated derivatives yielded a characteristic pattern of isotopic doublet signals, which was a highly diagnostic fingerprint for this group of compounds.

The main fragmentation process of CINP was similar to those observed for NP. The predominant reaction was the side chain fragmentation that resulted in sequential loss of 14 Da, with the most abundant fragments at  $m/z$  167 for  $^{35}\text{Cl}$  and  $m/z$  169 for  $^{37}\text{Cl}$  with the



**Figure 4.** CID spectra and the proposed fragmentation pattern of (a) CINPE<sub>1</sub>C (precursor ions *m/z* 311 and 313) and (b) BrNPE<sub>1</sub>C (precursor ions *m/z* 355 and 357) obtained at collision energy of 30 eV. Note: The exact branching of the alkyl chain is unknown and the alkyl isomer shown here is just one of several possibilities.

relative ratio of intensities of 3.03 (Figure 3a). Fragment corresponding to [Cl]<sup>−</sup> was produced only when sufficient collision energy was applied. The intensity of this ion was not very pronounced, but nevertheless remained useful for the identification of chlorinated NP.

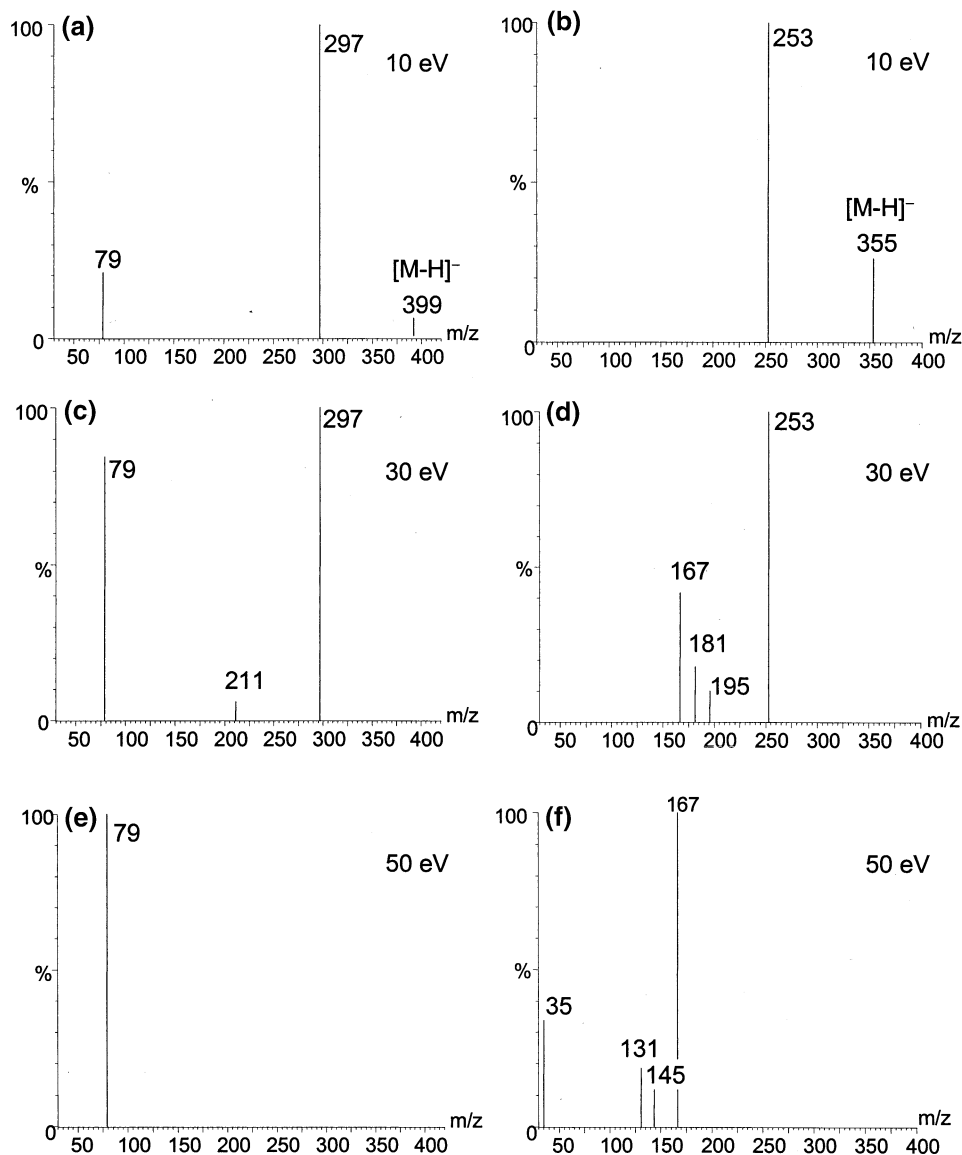
The brominated NP showed a markedly different fragmentation pathway (Figure 3b). At collision energy of 40 eV the product ion spectra of BrNP (*m/z* 297/299) yielded intense signals at *m/z* 79 and 81 corresponding to the [Br]<sup>−</sup> (ratio of isotopes 1.02), while the fragmentation of the side chain was suppressed and resulted just in a low-intensity fragment at *m/z* 211/213 produced after the loss of C<sub>6</sub>H<sub>14</sub>.

For halogenated NPE<sub>1</sub>Cs and NPE<sub>2</sub>Cs (Figure 4) the predominant reaction was loss of CH<sub>2</sub>COO and CH<sub>2</sub>CH<sub>2</sub>OCH<sub>2</sub>COO, respectively, that resulted in intense signals at *m/z* 253/255 for CINPECs and *m/z* 297/299 for BrNPECs. Further fragmentation yielded the same ions as described above for halogenated NPs, i.e., [<sup>79</sup>Br]<sup>−</sup> and [<sup>81</sup>Br]<sup>−</sup> for brominated NPECs and *m/z* 167/169 and *m/z* 181/183 for CINPECs. Such difference in the mechanism of fragmentation of chlorinated and brominated compounds is presumably the consequence of the lower energy of a Br–C<sub>6</sub>H<sub>5</sub> (benzene) bond compared to a Cl–C<sub>6</sub>H<sub>5</sub> bond.

BrNPECs yielded [Br]<sup>−</sup> at low collision energies (10 eV), as shown in Figure 5a for BrNPE<sub>2</sub>C, while

CINPECs produced [Cl]<sup>−</sup> only at higher collision energy (50 eV) (Figure 5f). At collision energy of 50 eV the *m/z* 79/81 ion was the sole fragment ion observed for BrNPE<sub>2</sub>C, while chlorinated analog showed, in addition to *m/z* 167/169, fragments at *m/z* 131 and *m/z* 145. These two fragments, observed as product ions of both <sup>35</sup>CINPE<sub>2</sub>C and <sup>37</sup>CINPE<sub>2</sub>C, respectively, did not show characteristic isotopic pattern and were tentatively assigned as fragments formed by the cleavage of ethoxy-carboxylated moiety, chlorine and by the cleavages in the alkyl moiety.

From the observed fragmentation pathways the dominant dissociation reaction channels were chosen (Table 1). The cone voltage of each transition was optimized to a value that gave the maximum abundance of the precursor ion, whereas the collision energy was set to a value that resulted in the maximal intensity of the product ion. Generally, deprotonated molecules were chosen as precursor ions, with the exception of NPE<sub>2</sub>C and halogenated NPE<sub>2</sub>Cs. Even at low cone voltages (5–10 V), in-source CID was obtained for these compounds and their spectra displayed low abundance of deprotonated molecules. Thus, for these compounds the most intense MRM channels were those monitoring the fragmentation of “the first generation” products (e.g., 219 → 133 for NPE<sub>2</sub>C, 297/299 → 79/81 for BrNPE<sub>2</sub>C, and 253/255 → 167/169 for CINPE<sub>2</sub>C).



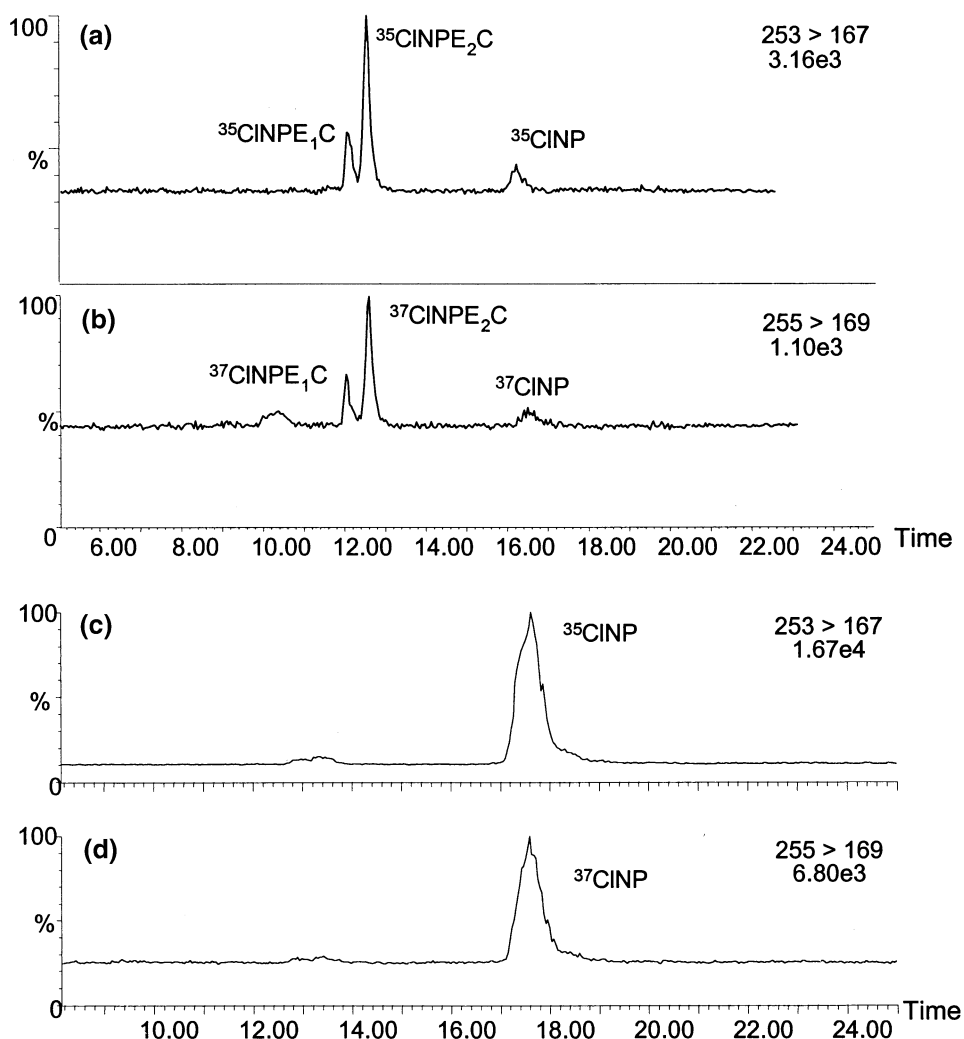
**Figure 5.** Product ion scans of XNPE<sub>2</sub>C obtained at 10, 30, and 50 eV: (a), (c), and (e) <sup>79</sup>BrNPE<sub>2</sub>C (precursor ion *m/z* 399); (b), (d), and (f) <sup>35</sup>ClNPE<sub>2</sub>C (precursor ion *m/z* 355).

Positive identification criterion of the target compounds was based on: (1) LC retention time of the analyte compared to that of a standard ( $\pm 2\%$ ), (2) the ratio of abundances of two specific precursor ion  $\rightarrow$  product ion transitions (within 10% of the ratios obtained for the standard). Additionally, for halogenated compounds a highly diagnostic criteria was the abundance ratio of characteristic isotopes ( $^{79}\text{Br}:$  $^{81}\text{Br} = 1.02$ ;  $^{35}\text{Cl}:$  $^{37}\text{Cl} = 3.03$ ) and for each transition reaction two specific channels, corresponding to two isotopes, were monitored and the ratio of their abundances calculated.

High selectivity of the MS-MS detection of halogenated nonylphenolic compounds is shown in Figures 6 and 7, which display extracted MRM chromatograms for chlorinated and brominated compounds, respectively, detected in water and sludge samples from

DWTP of Barcelona. A specificity of MRM mode permitted identification and quantification of halogenated NPECs, thus obviating isobaric interference of CINPE<sub>1</sub>C and C<sub>11</sub>LAS (both having a base ion at *m/z* 311) observed using a LC-MS in selected ion monitoring (SIM) mode [6]. Note that broad and, in some cases multiple peaks, observed, e.g., for brominated compounds (Figure 7), reflect the multitude of isomers arising from the different branching of the nonyl entity and probably co-existence of different isomers of the benzene ring, i.e., 2-NP, which makes up to 10% of NP in industrial blends, and consequently different position of bromine and chlorine, respectively.

Other MS-MS modes, such as the precursor ion scan and neutral loss scan, were found to be less useful MS/MS scanning modes as they were 5 to 10 times less



**Figure 6.** Detection of chlorinated nonylphenolic compounds in prechlorinated water [traces (a) and (b)] and flocculation sludge [traces (c) and (d)] from Barcelona drinking water treatment plant. (MRM mode: 253 → 167 and 255 → 169 for detection of  $^{35}\text{Cl}$  and  $^{37}\text{Cl}$ -nonylphenolic compounds, respectively).

sensitive than product ion MS-MS. The precursor ion scan of the  $m/z$  133 yielded the  $[\text{M} - \text{H}]^-$  ions of the respective NP, NPE<sub>1</sub>C, and NPE<sub>2</sub>C, together with small “isotopic” peaks one to several mass units higher. However, an acceptable mass chromatographic response was only obtained for nonylphenolic compounds, when present at concentrations higher than 0.5  $\mu\text{g/L}$ . For less abundant halogenated compounds precursor ion scan of  $m/z$  79/81 for brominated compounds and precursor ion scans of  $m/z$  167/169 and 35/37 for chlorinated compounds were not applicable to real-world samples due to low sensitivity.

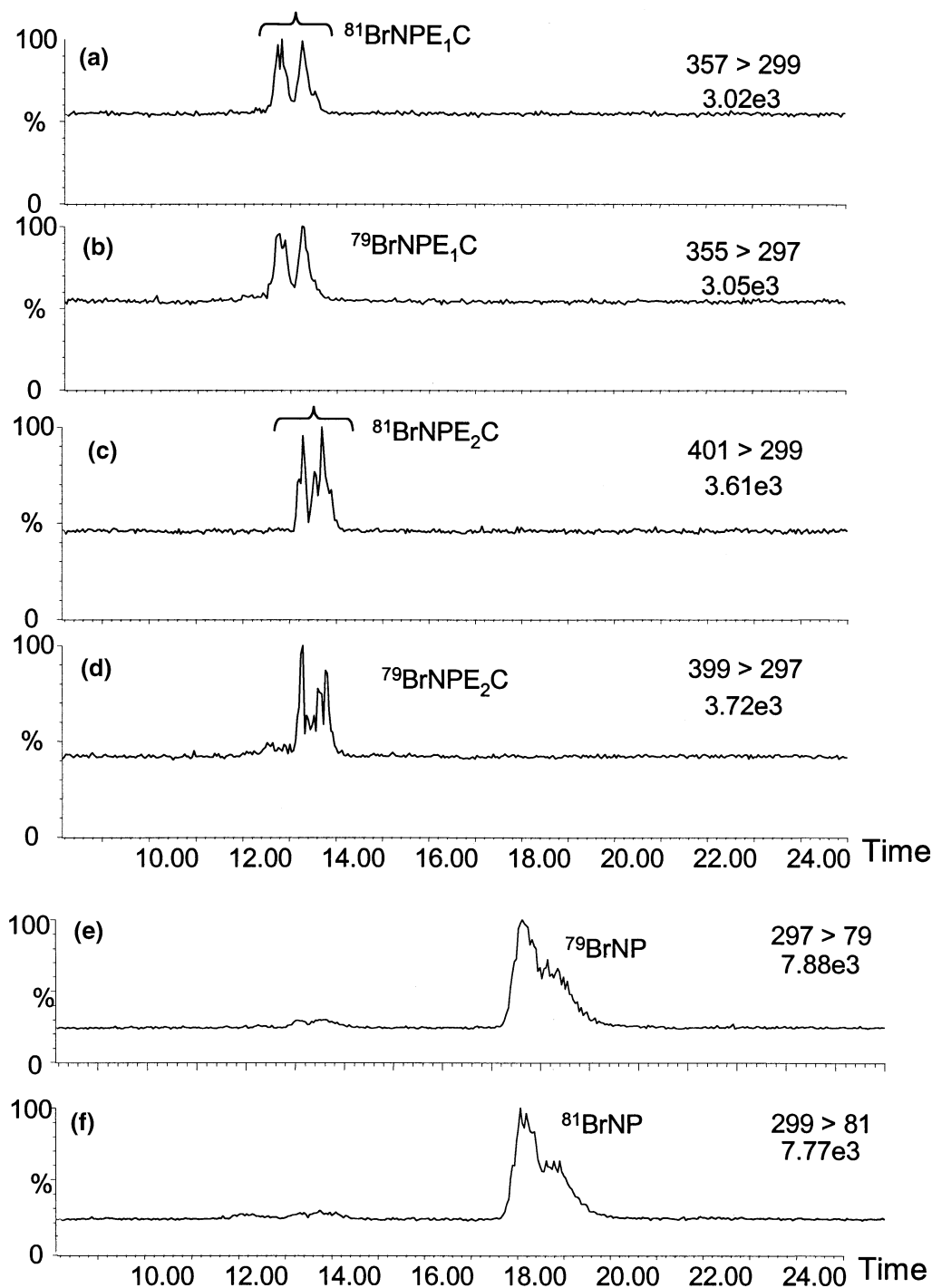
### Quantitative Analysis

**Matrix effect.** One of the problems to be solved when analyzing trace organics in complex matrices by LC-MS-(MS) is suppression of the analyte signal caused by high concentration of matrix components. The use of an

internal standard can compensate, over a limited retention time window, for signal irreproducibility and correct quantitative data. However, it cannot compensate for the overall sensitivity reduction. Another approach to cope with matrix effect and to avoid an erroneous quantitation is aimed at the reduction of matrix components prior to the LC-MS-(MS) analysis applying a selective extraction and improved sample clean-up. Although the later strategy is laborious, it is still the most direct mean to avoid the loss of sensitivity.

Analytical procedure (SPE for water samples and PLE with SPE clean-up for sludge samples), applied in this work, yielded rather clean extracts that produced low background MS noise, as described in details elsewhere [24]. The extent of ion suppression was checked by adding an internal standard (4-nonyloxy benzoic acid) to all extracts and monitoring the transition reaction from  $m/z$  263  $[\text{M} - \text{H}]^-$  to  $m/z$  92 (retention time  $t_R = 14.7$  min). In comparison to the signal





**Figure 7.** Detection of brominated nonylphenolic compounds in prechlorinated water [traces (a)–(d)] and flocculation sludge [traces (e) and (f)] from Barcelona drinking water treatment plant. MRM mode: (a) 357 → 299 and (b) 355 → 297 for  $\text{BrNPE}_1\text{C}$ ; (c) 401 → 299 and (d) 399 → 297 for  $\text{BrNPE}_2\text{C}$ ; (e) 297 → 79 and (f) 299 → 81 for  $\text{BrNP}$ .

intensity obtained in a standard solution (methanol), the flocculation sludge was the only sample that showed a reduced value (limited to 10 to 15% of ion suppression for all analyzed samples), while less complicated matrices (river water and drinking water) showed negligible loss of sensitivity.

**Validation Parameters.** Recoveries obtained using LC-MS-MS (MRM) were similar to those previously reported for LC-MS (SIM) [6, 24]. SPE of target analytes from water samples combined with LC-MS-MS detection yielded recoveries from 72 to 98% (see Table 2), whereas for solid samples PLE followed by SPE extract

**Table 2.** Instrumental detection limits ( $LOD_{inst}$ ) of LC-MS and LC-MS-MS, method detection limits ( $LOD_{method}$ ), and recoveries of combined SPE-LC-MS-MS for the analysis of target compounds in water samples and PLE-LC-MS-MS for solid samples

Compound	$LOD_{inst}$ (pg injected)		$LOD_{method}$		Recovery, % (RSD, $n = 3$ )	
	LC-MS (SIM)	LC-MS-MS (MRM)	Water samples (ng/L) <sup>a</sup>	Sludge samples ( $\mu$ g/kg) <sup>b</sup>	Water samples SPE-LC-MS-MS	Sludge samples PLE-LC-MS-MS
NP	60	10	1	0.5	85 (5)	81 (9)
XNP	50	15	2	1.0	72 (7) CINP 73 (7) BrNP	60 (14) CINP 64 (11) BrNP
NPE <sub>1</sub> C	100	30	2	1.5	98 (5)	78 (8)
XNPE <sub>1</sub> C	150	50	2	1.5	90 (6) CINPE <sub>1</sub> C 92 (7) BrNPE <sub>1</sub> C	71 (12) CINPE <sub>1</sub> C 75 (9) BrNPE <sub>1</sub> C
NPE <sub>2</sub> C	100	30	2	1.5	88 (4)	68 (10)
XNPE <sub>2</sub> C	200	50	2	1.5	84 (6) CINPE <sub>2</sub> C 79 (7) BrNPE <sub>2</sub> C	n.d.

<sup>a</sup>SPE preconcentration factor 1000; 20 MRM channels (for other conditions see Experimental part).<sup>b</sup>PLE, preconcentration factor 2; 20 MRM channel. %n.d. not determined.

clean-up and LC-MS-MS detection resulted in recoveries of 60 to 81% of target compounds. The relative standard deviation ( $n = 3$ ) for water analysis was below 7% and for sludge below 14%. Intra-day variability of calibration curve slopes was minimal (<5%), while inter-day variability of daily calibration curve slopes over 1 week period ranged from 5 to 10% depending on the analyte.

The comparison of instrumental detection limits ( $LOD_{inst}$ ) obtained using LC-MS and LC-MS-MS in SIM and MRM mode, respectively, and method detection limits ( $LOD_{method}$ ) (MRM mode) are shown in Table 2. The LODs were based on the peak-to-peak noise of the baseline near the analyte peak obtained by analyses of a standard solution ( $LOD_{inst}$ ) and real-samples ( $LOD_{method}$ ), respectively, and on minimal value of signal-to-noise ratio of 3. The method LODs refer to SPE-LC-MS-MS procedure for water samples, based on a preconcentration factor of 1000, and PLE-LC-MS-MS for sludge samples (preconcentration factor 2). Values of  $LOD_{inst}$  obtained by LC-MS-MS were approximately 3–6 times lower than by LC-MS (SIM). The detection limits of the method proposed here for the analysis of target compounds in water samples fell down to 1–2 ng/L and in sludge samples ranged from 0.5 to 1.5

$\mu$ g/kg, which is significant improvement in comparison to LODs reported previously for GC-MS [18] and LC-MS [6].

### Analysis of Real Samples

The feasibility of LC-MS-MS method for the analysis of halogenated and non-halogenated NP and NPECs was evaluated by analyzing samples from DWTP Sant Joan Despí in Barcelona. The waterworks, with the daily production of 300,000 m<sup>3</sup> of drinking water, are situated at the Llobregat River downstream of a densely industrialized area. The whole river basin receives effluents from more than 30 WWTPs, which results in poor quality of the water entering to DWTP. Moreover, the specificity of the Llobregat River is a high concentration of bromide ions (average 0.7 mg/L, maximum 1.2 mg/L in 2000) [25] that arises from salt mine activities in the upper course of the river. As a consequence, it was already reported that treated (tap) water contains rather high concentration of brominated disinfection by-products [26].

NP and NPECs were found in the Llobregat River water entering the water treatment plant in concentrations of 1.5, 2.0, and 2.9  $\mu$ g/L for NP, NPE<sub>1</sub>C and the

**Table 3.** Concentrations found DWTP (Barcelona, Spain) samples

Compound	Concentration in water (ng/L)						Flocculation sludge ( $\mu$ g/kg)
	Raw water	Prechlorination	Sand filter	Ozonation	Carbon filter	Chlorination	
NP	1500	240	220	250	160	92	150
NPE <sub>1</sub> C	2000	1640	1600	65	30	12	7.0
NPE <sub>2</sub> C	2900	1960	2200	76	35	10	20
BrNP	n.d.	17	19	<5	n.d.	n.d.	105
CINP	n.d.	9	12	<5	n.d.	n.d.	145
BrNPE <sub>1</sub> C	n.d.	120	130	57	21	<5	n.d.
CINPE <sub>1</sub> C	n.d.	14	19	13	5	<5	n.d.
BrNPE <sub>2</sub> C	n.d.	316	353	217	52	10	13
CINPE <sub>2</sub> C	n.d.	45	60	26	<5	<5	7.5

n.d. - not detected

most abundant NPE<sub>2</sub>C, respectively, whereas their halogenated analogs were not detected as expected (Table 3). The following treatment process consists of prechlorination, flocculation with aluminum sulfate, sand filtration, groundwater dilution, ozonation, granular activated carbon filtration and a final chlorination. During the prechlorination steps, NP and NPECs were found to transform to halogenated derivatives. Both, brominated and chlorinated NP and NPECs were detected in prechlorinated water in concentrations up to 315 ng/L, being BrNPE<sub>2</sub>C the most abundant specie. The main part of these compounds, as well as of their precursors were removed by ozonation and subsequent activated carbon filtration. After final chlorination, in the treated water leaving the DWTP non-halogenated compounds were detected at trace levels (85, 12, and 10 ng/L for NP, NPE<sub>1</sub>C, and NPE<sub>2</sub>C, respectively) indicating the efficient removal of these compounds (95% of NP and more than 99% of acidic metabolites). Halogenated derivatives were identified but their concentrations never exceeded 10 ng/L.

The flocculation sludge (daily production 30,000 kg dry matter) was found to accumulate hydrophobic halogenated compounds and their precursors. NP, brominated and chlorinated NPs were found in concentrations of 150, 105, and 145 µg/kg, respectively. Acidic, polar metabolites were found in concentrations up to 20 µg/kg.

## Conclusions

This work presents for the first time the application of liquid chromatography-tandem mass spectrometry to the analysis of halogenated nonylphenolic compounds and their precursors. MS-MS study of target compounds showed characteristic fragmentation pattern for each group of compounds that provided sufficient structural information and permitted the election of specific transition channels for their identification.

In addition to the advantages related to the quantification in MRM mode (high sensitivity and selectivity) the method allowed determination of isobaric target compounds (e.g., BrNPE<sub>1</sub>C and ClNPE<sub>2</sub>C) and elimination of interference of co-eluting isobaric no-target compounds (e.g., C<sub>11</sub>LAS). The method yielded very low detection limits: low ng/L level in water samples and low µg/kg level in sludge samples, thus providing a reliable and robust tool that can be used for routine analysis of estrogenic NP, NPECs, and their halogenated derivatives in aqueous and solid samples.

## Acknowledgments

The work has been supported by the EU Project P-THREE (EVK1-CT-2002-00116) and by the Spanish Ministerio de Ciencia y Tecnología (PPQ2002-10945-E). AD acknowledges a Ph.D. fellowship from Fundació Agbar. MP acknowledges the "Ramon y Cajal" contract from the Spanish Ministry of Science and Technol-

ogy. The authors thank Merck for supplying the SPE cartridges and LC columns.

## References

- Ahel, M.; Giger, W.; Koch, M. Behavior of Alkylphenol Polyethoxylate Surfactants in the Aquatic Environment. I. Occurrence and Transformation in Sewage Treatment. *Water Res.* **1994**, *28*, 1131–1142.
- Jonkers, N.; Knepper, T. P.; de Voogt, P. Aerobic Biodegradation Studies of Nonylphenol Ethoxylates in River Water Using Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *Environ. Sci. Technol.* **2001**, *35*, 335–340.
- Di Corcia, A.; Constantino, A.; Crescenzi, C.; Marinoni, E.; Samperi, R. Characterization of Recalcitrant Intermediates from Biotransformation of the Branched Alkyl Side Chain of Nonylphenol Ethoxylate Surfactants. *Environ. Sci. Technol.* **1998**, *32*, 2401–2409.
- Di Corcia, A.; Cavallo, R.; Crescenzi, C.; Nazzari, M. Occurrence and Abundance of Dicarboxylated Metabolites of Nonylphenol Polyethoxylate Surfactants in Treated Sewages. *Environ. Sci. Technol.* **2000**, *34*, 3914–3919.
- Ventura, F.; Figueras, A.; Caixach, J.; Espadaler, I.; Romero, J.; Guardiola, J.; Rivera, J. Characterization of Polyethoxylated Surfactants and Their Brominated Derivatives Formed at the Water Treatment Plant of Barcelona by GC/MS and FAB Mass Spectrometry. *Water Res.* **1988**, *22*, 1211–1217.
- Petrovic, M.; Diaz, A.; Ventura, F.; Barceló, D. Simultaneous Determination of Halogenated Derivatives of Alkylphenol Ethoxylates and Their Metabolites in Sludges, River Sediments, Surface, Drinking and Waste Waters by Liquid Chromatography-Mass Spectrometry. *Anal. Chem.* **2001**, *73*, 5886–5895.
- Ventura, F.; Caixach, J.; Figueras, A.; Espadaler, I.; Fraisse, D.; Rivera, J. Identification of Surfactants in Water by Fast Atomic Bombardment Mass Spectrometry. *Water Res.* **1989**, *23*, 1191–1203.
- Ventura, F.; Caixach, J.; Romero, J.; Espadaler, I.; Rivera, J. New Methods for the Identification of Surfactants and Their Acidic Metabolites in Raw and Drinking Water: FAB MS and MS/MS. *Water Sci. Tech.* **1992**, *25*, 257–264.
- Reinhard, M.; Goodman, N.; Mortelmans, K. E. Occurrence of Brominated Alkylphenol Polyethoxy Carboxylates in Mutagenic Wastewater Concentrates. *Environ. Sci. Technol.* **1982**, *16*, 351–362.
- Fujita, M.; Ike, M.; Mori, K.; Kaku, H.; Sakaguchi, Y.; Asano, M.; Maki, H.; Nishihara, T. Behavior of Nonylphenol Ethoxylates in Sewage Treatment Plants in Japan—Biotransformation and Ecotoxicity. *Water Sci. Technol.* **2000**, *42*, 23–30.
- Servos, M. R. Review of the Aquatic Toxicity, Estrogenic Responses, and Bioaccumulation of Alkylphenols and Alkylphenol Polyethoxylates. *Water Qual. Res. J. Canada* **1999**, *34*, 123–177.
- Ahel, M.; Giger, W. Partitioning of Alkylphenols and Alkylphenol Polyethoxylates Between Water and Organic Solvents. *Chemosphere* **1993**, *26*, 1471–1478.
- Jobling, S.; Sheahan, D.; Osborne, J. A.; Matthiessen, P.; Sumpter, J. P. Inhibition of Testicular Growth in Rainbow Trout (*Oncorhynchus Mykiss*) Exposed to Estrogenic Alkylphenolic Chemicals. *Environ. Toxicol. Chem.* **1996**, *15*, 194–202.
- Jobling, S.; Sumpter, J. P. Detergent Components in Sewage Effluent are Weakly Estrogenic to Fish: An in Vitro Study Using Rainbow Trout (*Oncorhynchus mykiss*) hepatocytes. *Aquat. Toxicol.* **1993**, *27*, 361–372.
- Maki, H.; Okamura, H.; Aoyama, I.; Fujita, M. Halogenation and Toxicity of the Biodegradation Products of a Nonionic

- Surfactant, Nonylphenol Ethoxylates. *Environ. Toxicol. Chem.* **1998**, 17, 650–654.
16. Requena, V.; Petrovic, M.; Garcia-Reyero, N.; Barceló, D.; Hansen, P. D.; Piña, B. Estrogenic Potential of Halogenated Derivatives of Nonylphenol Ethoxylates and Carboxylates; unpublished.
  17. Ventura, F.; Fraisse, D.; Caixach, J.; Rivera, J. Identification of [(Alkylloxy)polyethoxy] Carboxylates in Raw and Drinking Water by MS/MS and Mass Determination Using FAB and Nonionic Surfactant as Internal Standard. *Anal. Chem.* **1991**, 63, 2095–2099.
  18. Diaz, A.; Ventura, F.; Galceran, M. T. Development of a Solid-Phase Microextraction Method for the Determination of Short-Ethoxy-Chain Nonylphenols and Their Brominated Analogs in Raw and Treated Water. *J. Chromatogr. A* **2002**, 963, 159–167.
  19. Diaz, A.; Ventura, F.; Galceran, M. T. Simultaneous Determination of Estrogenic Short Ethoxy Chain Nonylphenols and Their Acidic Metabolites in Water by an In-Sample Derivatization/Solid Phase Microextraction Method. *Anal. Chem.* **2002**, 74, 3869–3876.
  20. Ferguson, P. L.; Iden, C. R.; Brownawell, B. J. Analysis of Alkylphenol Ethoxylate Metabolites in the Aquatic Environment Using Liquid Chromatography-Electrospray Mass Spectrometry. *Anal. Chem.* **2000**, 72, 4322–4330.
  21. Ferguson, P. L.; Iden, C. R.; Brownawell, B. J. Distribution and Fate of Neutral Alkylphenol Ethoxylate Metabolites in a Sewage-Impacted Urban Estuary. *Environ. Sci. Technol.* **2001**, 35, 2428–2435.
  22. Hao, C.; Croley, T. R.; March, R. E.; Koenig, B. G.; Metcalfe, C. D. Mass Spectrometric Study of Persistent Acid Metabolites of Nonylphenol Ethoxylate Surfactants. *J. Mass Spectrom.* **2000**, 35, 818–830.
  23. Stokker, G. E.; Daena, A. A.; Desolms, S. J.; Schultz, E. M.; Smith, R. L.; Cragoe, E. J.; Baer, J. E.; Ludden, C. T.; Russo, H. F.; Scriabine, A.; Sweet, C. S.; Watson, L. S. 2-(Aminomethyl)Phenols, a New Class of Saluretic Agents. *J. Med. Chem.* **1980**, 23, 1414–1427.
  24. Petrovic, M.; Lacorte, S.; Viana, P.; Barceló, D. Pressurized Liquid Extraction Followed by LC-MS for the Determination of Phenolic Xenoestrogens in River Sediment. *J. Chromatogr. A* **2002**, 959, 15–23.
  25. AGBAR, Aigues de Barcelona, Internal Report. 2001.
  26. Cancho, B.; Ventura, F.; Galceran, M. T. Behavior of Halogenated Disinfection By-Products in the Water Treatment Plant of Barcelona, Spain. *Bull. Environ. Contam. Toxicol.* **1999**, 63, 610–617.